II. Patentability Arguments

A. The Rejection Under 35 U.S.C. § 102(e) Should Be Withdrawn

The Examiner rejected pending claims 45-54 under 35 U.S.C. 102(e) allegedly as being anticipated by U.S. Patent No. 5,223,409 to Ladner et al. (*Ladner*).

The Examiner characterized the pending claims as being drawn to recombinant cells comprising phagemid comprising: 1) phage origin of replication; 2) gene III coat protein surface component; 3) gene encoding for "a specific binding pair" fused to gene III. *1st paragraph at page 5 of the office action.*

Applicants respectfully bring to the Examiner's attention that in addition to the elements listed by the Examiner above, the pending claims specifically recite that "the gene III coat protein surface component encoding nucleic acid and the origin of replication being the only nucleic acid in the phagemid derived from filamentous bacteriophage."

The Examiner characterized *Ladner* as

> "teaching phagemid vectors particularly phagemid vectors pBluescript K/S and pGEM-3Zf (only ori from filamentous bacteriophage; please refer to column 76; lines 55-67; column 77; lines 1-4; column 106) wherein the construct comprising gIII-binding domain would be inserted into the multiple cloning site for phage display (i.e. plasmid would then contain only ori and gIII of filamentous bacteriophage; refer to columns 53-59, section IV.B). Further, *Ladner* states that while certain phagemids are not preferred for their purposes (i.e. controlling mutations via random mutagenesis of a limited number of predetermined codons; column 1, lines 40-52) because conifection could lead to genetic recombination (i.e. non-controlled mutation), phagemids are suitable for developing a gene that causes a binding domain to appear on the surface of phage-like genetic package (please refer to paragraph spanning columns 76 and 77)."

Applicants respectfully bring to the Examiner's attention that these passages do not disclose a recombinant cell comprising a phagemid in which "the gene III coat protein surface component encoding nucleic acid and the origin of replication being the only nucleic acid in the phagemid derived from filamentous bacteriophage" as required by the pending claims.

The Examiner alleges that *Ladner* discloses in column 106 the use of pGEM-3Zf, which according to the Examiner comprises only of ori and would be used for insertion of gIII. However, this is not what the *Ladner* disclosure is.

Ladner Column 106 begins with the following title:

"Construction of the VIII-signal-sequence::bpti::mature-VIII-coat-protein display vector." It is under this title that **pGEM-3Zf is used as the starting material** to build the VIII-signal-sequence::bpti::mature-VIII-coat-protein display vector. Thus, the *Ladner* construct comprises gVIII and the phagemid recited by the pending claims does not comprise gVIII.

The present invention as claimed employs a gene III capsid protein not the gene VIII coat protein and thus it is novel and not anticipated by the vector construct described in column 106 of *Ladner* for this reason in itself. Furthermore, it is explicit in column 106 of *Ladner* that the signal peptide employed is a filamentous bacteriophage gene VIII signal peptide, the construct created being:

viii-signal-sequence::bpti::mature-viii-coat-protein

The gene VIII signal peptide is a nucleotide sequence derived from filamentous bacteriophage that is other than an origin of replication and a nucleotide sequence encoding a gene III capsid protein and the signal peptide is not a component of the viral capsid protein, being cleaved away. The presence of an additional filamentous bacteriophage component in addition to the only two permitted in the present claim (origin of replication and a nucleotide sequence encoding a gene III protein) prevents *Ladner* from anticipating the present invention.

The Examiner also refers to Section IV.B as the alleged *Ladner* disclosure of "the plasmid would then contain only ori and gIII of filamentous bacteriophage." However, Section IV.B entitled "Phages for use as GPs" focuses exactly on what is stated in its title – phages. It discloses how filamentous phage, i.e. M13, is of particular interest. *See* lines 37-38 of column 54. This is a disclosure of an entire phage (M13) to be used as a GP. Nowhere does Section IV.B disclose "the plasmid would then contain only ori and gIII of filamentous bacteriophage" as stated by the Examiner.

Further, as discussed in Applicants' previous response of March 14, 2008, *Ladner's* stated concern is that if a helper phage is used there will be recombination between different DNA's encoding displayed molecules and thus that the genotype/phenotype connection would be lost. *See* lines 63 to 68 in column 76. The Examiner interpreted the *Ladner* statement as applicable to situations with controlled mutagenesis only. However, *Ladner* does not limit its statement to controlled mutagenesis only. It simply states "Phagemids are not preferred for our purposes." This would apply whatever the population source may be, including from controlled mutagenesis or otherwise. When *Ladner* says that "Phagemids may be entirely suitable for developing a gene that causes an IPBD to appear on the surface of phage-like genetic packages" this refers to making a phagemid with the full genome of M13, as discussed in detail in Applicants' previous response of March 14, 2008. The disclosure in *Ladner* did not use Bluescript K/S that is mentioned in column 76.

In conclusion, in view of the differences between the *Ladner* disclosure and the subject-matter as recited by the pending claims, Applicants respectfully submit that *Ladner* cannot properly anticipate any of the pending claims as a matter of law and, therefore, the rejections of the claims over *Ladner* should be withdrawn.

B. The Rejection Under 35 U.S.C. § 103(a) Should Be Withdrawn

Claims 45-54 stand rejected under 35 U.S.C. 103(a) allegedly as being obvious over Parmley (Gene. Vol. 73: 305-318; 1988; *Parmley*), in view of Ladner (WO 88/06630; *Ladner WO*) and Geider (Gene. Vol. 33: 341-349; *Geider*) and if necessary in view of Mead (Biotechnology. Vol.10: 85-102; 1988; *Mead*).

In response to the previous office action, Applicants provided comments on *Smith, Parmley and Ladner WO. See* Applicants' response of March 14, 2008. As suggested in the Office Action of June 12, 2008, the Examiner recognizes that *Parmley* combined with *Ladner WO* do not render the present invention obvious and as explained in more detail below, newly cited *Geider* and *Mead* add nothing of relevance to the *Parmley/Ladner WO* combination. As further discussed below, no *prima facie* case of obviousness has been established based on the *Parmley/Ladner WO/Geider/Mead* combination because the references even when combined, *inter alia,* do not describe or

suggest that it is possible to display functional, folded protein domains on the surface of filamentous bacteriophage particles.

a) Parmley's denatured peptide is not a functional specific binding domain

Claim 54 recites that "each [displayed] member of the specific binding pair comprises a functional specific binding domain." Applicants discussed at length in their previous response the definition of the functional specific binding domain and that it means "the same or closely analogous to its native configuration." *See* page 6 of the Applicants' response of March 14, 2008 and also page 30, lines 19-29 of the specification.

The Examiner characterized *Parmley* as teaching phage displaying antigens, which are screened for specific antibodies. The Examiner further asserts at page 8 of the Office Action that *Parmley* "teaches inserting a nucleic acid encoding for an antigen (such as fragments of β-gal protein) (e.g. p. 307, col. 1, para 1), which the antigen reads on the "one member of a specific binding pair" because the antigen binds to a specific antibody." Thus, the Examiner's position is that the *Parmley* β-gal fragment is "a member of the specific binding pair compris[ing] a functional specific binding domain." For the reasons provided below, this conclusion is in error and the *Parmley* β-gal fragment is not an equivalent of a functional specific binding domain.

Parmley discusses display of a 335bp fragment of beta-galactosidase corresponding to nucleotides 861-1195 in the gene sequence. This fragment of a gene encodes 112 amino acids of a much larger 380 amino acid domain. Thus, an incomplete domain is displayed, and that which is displayed has none of the functions of beta-galactosidase. It is unknown whether the three-dimensional structure of this 112 amino acid insert is the same as in the full domain, although it would be surprising if it were since interactions with other parts of the domain would be expected to affect folding and hence structure. For recognition by the rabbit polyclonal anti-beta galactosidase antibody used by *Parmley* in their 'biopanning' procedure, no folding of the 112 amino acid insert would have been required since it would be expected that the polyclonal antiserum would contain antibodies against linear amino acid epitopes which could be recognised in either denatured or folded beta-galactosidase.

To summarize, the *Parmley* fragment is a denatured peptide and not a functional specific domain as recited by the pending claims. The fact that this fragment is recognized by a polyclonal antibody is not evidence that the fragment was folded into a functional domain because polyclonal antibodies recognize denatured polypeptides.

The Examiner states at page 11 of the Office Action that "*Parmley* has shown that different proteins with different sequences can be displayed in phage." This conclusion is in error because "protein" is the term of art reserved for properly folded structures that form domains. *See* page 6 of Applicants' response of March 14, 2008. *Parmley* used a truncated, denatured peptide which did not represent a functional specific binding domain.

b) *Smith's peptide is not a functional specific binding domain*

Applicants bring to the Examiner's attention that while the Examiner does not cite *Smith* at page 8 of the Office Action as one of the references in the obviousness combination, the Examiner still cites *Smith* at page 9 of the Office Action where the Examiner admits that *Smith* does not teach using phagemid as recited by claim 54. *See* page 9 of the Office Action. Applicants respectfully bring to the Examiner's attention that all other pending claims depend on claim 54 and therefore, they also recite phagemid.

Further, *Smith* does nothing to remedy the deficiencies of *Parmley* because *Smith* also teaches only a display of a denatured peptide and not a functional domain. Specifically, *Smith* demonstrated the display of a 171bp Sau3A fragment of *E. coli* EcoRI endonuclease. Examination of the DNA sequence of EcoRI endonuclease (A.K. Newman *et al., J. Biol. Chem.* 2562131-2139 (1981)) indicates that this would correspond to amino acids 76-133 of the protein. The X-ray crystal structure (Y.C. Kim *et al., Science* 249:1307-1309 (1990)) indicates that this region would contain just two strands of a three strand anti-parallel beta-sheet which forms part of a larger five strand beta sheet structure. Thus, an incomplete and non-functional domain would have been displayed by Smith which would not have contained the amino acid sequences which are involved in binding of the protein to DNA (residues 103-241). The fragment of EcoRI endonuclease displayed on phage is recognized in the *Smith* paper by a **polyclonal** antiserum which would be expected to contain antibodies which would recognize **linear**

epitopes which did not need to be folded to achieve their native structure.

c) Ladner WO discloses only display in lambda phage and even this disclosure is non-enabling as to an antibody or any other functional specific binding domain

The Examiner states that *Ladner WO* teaches using phage (lambda phage) to display antibody fragments such as single chain antibodies (e.g. Abstract, pp. 2-3) and that the reference further teaches generating a large repertoire of genes encoding for single chain antibodies and displaying the antibodies on the surface of the phage (e.g. p. 4; Figure 3).

As discussed in detail in Applicants' response of March 14, 2008, lambda phage is different from filamentous bacteriophage recited by the pending claims. Lambda phage is a lytic phage and it is not a secreted bacteriophage, while filamentous bacteriophage is not lytic phage and it is a secreted bacteriophage.

The Examiner states at page 11 of the Office Action that *Ladner WO* has shown that "antibodies can be successfully displayed in phage." This assertion is in error because as explained in more detail below *Ladner WO* provided no experimental demonstration of antibody display at all. *Ladner WO* is not enabling for antibody display. Also, as discussed above that whatever data *Ladner WO* does have is only with Lambda phage which is not filamentous bacteriophage as recited by the pending claims.

A skilled person trying to follow *Ladner WO* and display "SCAD's" on the surface of lambda phage as taught by *Ladner WO* would have failed.

Lambda is a complete different kind of virus/phage from the filamentous bacteriophage used in the present invention. Bacteriophage lambda is not a secreted bacteriophage. Lambda is a lytic phage: attention is drawn to "Lambda II" (eds. Hendrix *et al.*) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1983: "A Beginner's Guide to Lambda Biology" (Arber) and "Tail Assembly and Injection" (Katsura).

There is no enabling disclosure in *Ladner WO* of the display of any antibody molecules on the surface of any microorganism, including phage lambda. "Phage λ" is assembled in the reducing environment of the cytoplasm of infected *E. coli* cells. *Ladner WO* recognizes the significance of the cysteines of antibodies in folding of functional

molecules: "reduced cysteines will greatly destabilize folding of a SCA" (page 7, third paragraph). Cysteines form disulphide bonds and, as *Ladner WO* says, "one would not expect disulfide bonds to form" in the reducing environment of the cell interior. There are two types of disulfide bridges in immunoglobulins. One is the conserved intrachain bridge, spanning about 60 to 70 amino-acid residues and found as a monotonous theme in every immunoglobulin domain. Buried deep between the opposing β-sheets, these bridges are shielded from solvent and ordinarily, can be reduced only in the presence of denaturing agents. As a general rule, without (stable) folding, an antibody molecule will not be functional; it will not bind antigen.

Ladner WO proposes a solution to this problem:

"*...reduced cysteines will greatly destabilize folding of a SCA. Therefore, to get proper folding of SCAD inside a cell, one mutates the SCAD gene to change all or some of the CYS's to SER, THR, ALA or GLY.*" [page 7, third paragraph].

In other words, *Ladner WO* teaches "in order to get the invention to work, all or some of the cysteines in the single chain antibody domain must be changed to SER, THR, ALA or GLY." However, there is evidence that by following these instructions and mutating cysteine residues of antibody fragments ("Fv" fragments, "*single chain Fv" ("scFv") fragments* and "Fab" fragments) to replace those residues with other amino acids stability is lost, and binding ability for antigen is lost as well.

Thus, no functional binding domain would have resulted and a skilled person would have failed in his efforts to achieve display of a functional, folded domain on the phage lambda surface. The ordinary skilled person would not, therefore, learn that it is possible to display functional, folded antibody domains on the surface of any organism, by following the teaching of *Ladner WO*. It teaches a problem with producing "SCADs" in the cytoplasm but, as discussed, only teaches one "solution," which "solution" has been shown to be inoperable. There is absolutely no suggestion in *Ladner WO* of any other solution, such as use of secreted filamentous bacteriophage.

To summarize so far, *the Parmley/Ladner WO combination* does not teach or suggest the display of functional binding domains as recited by the pending claims. *The Parmley/Ladner WO combination* does not teach or suggest the use of phagemid as recited by the pending claims. *The Parmley/Ladner WO combination* does not teach or

suggest the expression of a nucleic acid fragment encoding one member of a specific binding pair fused to a nucleic acid encoding a gene III coat protein surface component of a filamentous bacteriophage resulting in the display at the surface of bacteriophage, as recited by the pending claims.

d) *Geider and Mead disclose only cloning vectors*

The Examiner cited *Geider* and *Mead* at page 9 of the Office Action. The Examiner characterized the two references as teaching plasmids containing "the origin of replication from phage as the only DNA sequence from phage" (*Geider)* and various phage vectors including various phagemid that comprise only the origin of replication (*Mead). See* page 9 of the Office Action. Thus, the Examiner agrees that neither *Geider* nor *Mead* teach or suggest any display at the surface of bacteriophage, let alone a display of a functional domain. Neither do *Geider* and *Mead* teach or suggest the expression of a nucleic acid fragment encoding one member of a specific binding pair fused to a nucleic acid encoding a gene III coat protein surface component of a filamentous bacteriophage.

As previously explained in detail, the claims require the formation of folded protein domains on the surface of the particles. It was not obvious from *Parmley* with *Ladner WO* that this would be possible (*Parmley* being concerned with unfolded peptide fragments and *Ladner WO* with a completely different kind of bacteriophage – phage lambda which is not a secreted bacteriophage but is instead a lytic phage that forms within the very different environment within the cells).

Geider relates to use of plasmids containing phage origin of replication ("phagemids") as cloning vectors that "offer a convenient tool for subcloning of restriction fragments". *Mead* provides a review of various phage vectors including various phagemid, providing a convenient source for single stranded cloned DNA.

Subcloning and provision of single-stranded cloned DNA teach nothing about ability of filamentous bacteriophage particles to display functional specific binding domains. That which is unobvious from the combination of *Parmley* and *Ladner WO* (recognised by the Examiner) remains unobvious from the combination of *Parmley* and *Ladner* and *Geider,* with or without *Mead.* Neither of the new documents is concerned at all with what is actually contributed to the art by the present invention as claimed.

Neither document is concerned at all with any kind of display on the surface of bacteriophage particles, only cloning and expression of DNA within vectors.

Therefore, both *Geider* and *Mead* fail to teach or suggest the expression of a nucleic acid fragment encoding one member of a specific binding pair fused to a nucleic acid encoding a gene III coat protein surface component of a filamentous bacteriophage, as recited by the pending claims.

e) Conclusion: no prima facie case of obviousness has been established

In order to establish *a prima facie* case of obviousness, the Examiner is required to cite prior art references that when combined, teach or suggest each and every element recited by the pending claims. *See* MPEP, §2142. As discussed in detail above, *the Parmley/Ladner WO/Geider/Mead* combination does not disclose or suggest that it is possible to display functional, folded protein domains on the surface of filamentous bacteriophage particles. In light of this, no *prima facie* case of obviousness has been established and therefore, the rejection of the pending claims over the combination may be properly withdrawn; and withdrawal is respectfully requested.

C. Double Patenting Rejections

The Examiner rejected the pending claims based on the doctrine of nonstatutory obviousness-type double patenting over US patents 5,871,907; 5,858,657; 6,916,605; 7,063,943; 6,544,731; 6,521,404; 6,291,650; 6,225,447, 5,837,242 and 5,885,793. See page 11 of the Office Action. To overcome this rejection a terminal disclaimer may be submitted at the time that the pending claims become allowable but for the nonstatutory obviousness-type double patenting rejection. Applicants request that the submission of the terminal disclaimer be held in abeyance until the pending claims are deemed allowable but for the nonstatutory obviousness-type double patenting rejection over US patents 5,871,907; 5,858,657; 6,916,605; 7,063,943; 6,544,731; 6,521,404; 6,291,650; 6,225,447, 5,837,242 and 5,885,793.

III. Conclusion

In view of the above amendments and remarks, Applicants respectfully submit that the instant application is in good and proper order for allowance and early notification to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the instant application, the Examiner is encouraged to call the undersigned at the number listed below.

Respectfully submitted,

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